ALTERNATIVE BIOPOLYMERS IN EARLY EVOLUTION. E. Biondi[1,2], Z. Yang[1], L. Zhang[3], S. Dasgupta[4], J. A. Piccirilli[4], N. A. Leal[2] and S. A. Benner[1,2]. [1]Foundation for Applied Molecular Evolution, 13709 Progress Blvd., Alachua FL, 32615, [2]Firebird Biomolecular Sciences LLC, Alachua FL, [3]University of Florida, Gainesville FL, [4]University of Chicago, Chicago IL.

Introduction: One of the more discussed hypotheses for the origin of life is the "RNA first" model. This model postulates that organic molecular systems first gained access to Darwinism through a spontaneous prebiotic formation of RNA molecules that were able to generate replicates, with imperfections, where the imperfections were themselves replicable.

If this model were true, one would think that the key product that it proposes (an RNA replicase) would be easily obtained by modern prebiotic chemists who, after all, can access directed and prospective evolution, including the ability to order reagents with controlled purity, to avoid conditions that are obviously destructive, and to guide with facility the same process that, on early Earth, allegedly produced the replicase without guidance of any kind. To date, only a highly derived RNA ligase, developed in the Holliger lab, has come even close to being an RNA replicase [1].

The fact that it is so difficult to reproduce an RNA species central to the "RNA first" hypothesis suggests that we are missing something. Thus, many laboratories have sought alternative biopolymers that are both prebiotically accessible, and that also support Darwinism better than standard RNA, managing the rather low intrinsic catalytic ability of RNA as it is today found in terran biology, and the frequently reproduced experimental observation that it is easier to get nucleic acid molecules that catalyze the destruction of RNAs than nucleic acids that catalyze the synthesis of RNA.

This presentation will report experimental results with such nucleic acid-like biopolymers made from six different building blocks (Artificially Expanded Genetic Alphabet, or AEGIS). One of these is a C glycoside, a class of molecules that the Hud group showed might be accessible by simple aldol condensation of electron-rich heterocycles with carbohydrates [2]. These additional nucleotides carry functionality that chemical theory suggests might assist in binding and catalysis, perhaps even catalysis for the synthesis of RNA.

The presentation will briefly describe molecular biology for this artificial genetic system, including pipelines to synthesize its nucleoside triphosphates and phosphoramidites, procedures to synthesize a oligonucleotides, enzymes that copy the alien genetic system, and procedures that place the expanded, richer, genetic system under Darwinian selection pressure in the laboratory, with downstream sequencing and analysis to assess the sequelae of laboratory Darwinism.

These results have led to several discoveries. First, additional building blocks appeared to allow the system to adopt macro conformations different from, and additional to, those accessible with standard four letter nucleic acids. Further, it appears that the added information density by added letters provides options for the system to access specific binding confirmations. Further, although quantitative comparison is difficult, preliminary data suggests that the added functionality also allows the system to get tighter and more specific interactions between evolvable biopolymers and a target as it serves both genetic and phenotypic roles.

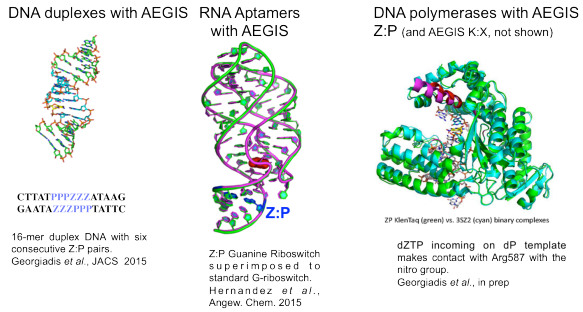

Figure 1. Structural biology of AEGIS.

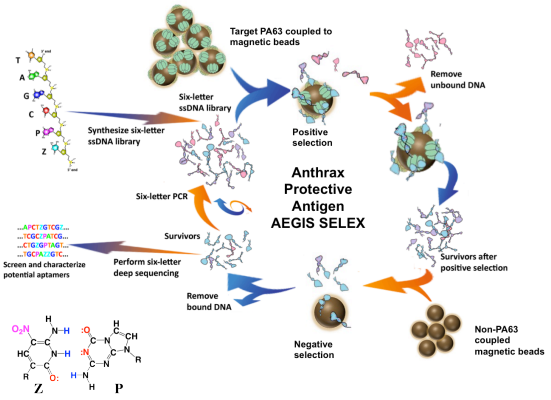

Figure 2. Anthrax Protective Antigen AEGIS-SELEX. (Biondi *et al.*, Nucleic Acids Res., 2016).

References: [1] Mutschler, H., Wochner, A., Holliger, P. (2015) Freeze–thaw cycles as drivers of complex ribozyme assembly. *Nat Chem 7*, 502-508. [2] Chen, M.C., Cafferty, B.J., Mamajanov, I., Ga´llego, I., Khanam, J., Krishnamurthy, R., and Hud, N.V. (2014) Spontaneous prebiotic formation of a **β**-ribofuranoside that self-assembles with a complementary heterocycle. *J Am. Chem. Soc* 136, 5640–5646.